It appears that Pref-1 protein expression in ducts may also mark undifferentiated pluripotent duct cells and be expressed transiently after Px. To test this hypothesis, semi quantitative RT-PCR analysis can be run for Pref-1 using the primers:

5'CCTTGTGCTGGCAGTCCTTTCC (SEQ ID NO:7)

3'TCTGTGAGGCTGACAATGTCTGC (SEQ ID NO:8)

for rat Pref-1 with a-tubulin for an internal control for samples from isolated common pancreatic ducts from rats 4, 12, 24 hrs and 2, 3, 7 days after Px and sham Px surgery as well as unoperated controls. Standardized conditions for linear amplification for this set of primers has already been prepared using techniques as in our previous studies. In parallel, immunostain for Pref-l/FA-1 can be performed using an anti-Pref-1 antibody. The advantage of the RT-PCR for the initial screening is that much information can be gleaned from a small amount of tissue or one experiment. The disadvantage is that heterogeneity is not accounted forand that only a few cells that are strongly positive for a gene may give a false interpretation. The second tier of analysis, immunostaining, overcomes these problems of interpretation of RT-PCR. Particular attention can be given to Pref-1 protein expression in the common pancreatic ducts and in the ductules in the focal areas of regeneration. If the expression pattern resembles that of PDX-1, then double immunostain for PDX-l (nucleus) and Pref-l (plasma membrane/cytoplasm) can be performed to confirm whether there is co-localization. It is unclear from the previous reports if Pref-1 is regulated at the transcriptional or posttranscriptional level. If it is, various mammalian members of the Notch family can be screened using RT-PCR.

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